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Original article

Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by *Punica granatum* Linn. leaves extract

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ABSTRACT

With an objective to develop Complementary and Alternative Medicine for the treatment of diabetic nephropathy, the present study investigated the protective effects of methanolic extract of *Punica granatum* leaves (MPGL) in streptozotocin-induced diabetic nephropathy. Diabetic nephropathy has become a leading cause of end stage renal failure worldwide. *P. granatum*, due to its anti-diabetic, anti-inflammatory and antioxidant activities may retard the progression of diabetic nephropathy. In this study, diabetes was induced by a single injection of streptozotocin (STZ, 45 mg/kg, i.p.) in rats. STZ-diabetic rats were treated with oral doses of MPGL (100, 200 and 400 mg/kg) for 8 weeks. At the end of the experimental period, body and kidney weight and blood glucose levels were determined. Serum and urine parameters were investigated. Antioxidant enzymes and lipid peroxide levels were determined in the kidney along with histopathological examination of the same. MPGL significantly increased body weight, lowered blood glucose levels and ameliorated kidney hypertrophy index in the STZ-diabetic rats. The extract also decreased the levels of creatinine, blood urea nitrogen, total cholesterol, triglycerides, advanced glycation end products and albumin in serum and urine, respectively. MPGL significantly increased the antioxidant parameters in the kidney. Histological evaluation revealed that MPGL treated STZ-diabetic rats demonstrated reduced vacuolar degeneration of tubules; periodic acid Schiff base (PAS) positivity staining intensity in glomeruli and basement membrane thickening. Present findings provide experimental evidence that MPGL has potential antioxidant, antihyperglycemic and anti-glycation activities which might be helpful in slowing the progression of diabetic nephropathy.

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1. Introduction

Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Sustained hyperglycemia is further associated with long term damage, dysfunction and failure of various organs and is a major factor in the development of many complications in patients with diabetes.[1,2] Moreover, diabetes is the most common cause of progressive kidney failure leading to dialysis or transplantation.[3] Nephropathy is reported to develop in 30–40% of patients with diabetes and has become a leading cause of end stage renal failure worldwide.[4,5] Diabetic nephropathy is characterized by structural as well as functional abnormalities.[6] Poor glycemic control and accumulation of advanced glycation end products (AGEs) play a significant role in the development of diabetic nephropathy. Furthermore, advanced glycation end products have been implicated in tissue damage associated with diabetic nephropathy. The clinical and pathological

Abbreviations: MPGL, methanolic extract of *Punica granatum* leaves; STZ, streptozotocin; AGEs, advanced glycation end products; GAE, gallic acid equivalent; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; IAEC, Institutional Animal Ethics Committee; BUN, blood urea nitrogen; DTNB, 5-5-dithiobis (2-nitrobenzoic acid); GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; H&E, hematoxylin and eosin; PAS, periodic acid Schiff base; MDA, malondialdehyde; ROS, reactive oxygen species.

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hallmarks of diabetic nephropathy include urinary albumin excretion[7] along with accumulation of extracellular matrix,[3] thickening of basement membranes, mesangial expansion, hypertrophy and glomerular epithelial cell (podocyte) loss within the glomeruli.[6] Patients with diabetic nephropathy have a progressive decline in glomerular function.[8] Antihypertensive agents, particularly those targeting the renin angiotensin system, such as angiotensin converting enzyme inhibitors, angiotensin receptor-1 antagonists are reported to be the most effective treatments for progressive diabetic nephropathy, to date. However, these treatments are not capable of preventing the onset of diabetic nephropathy.[6]

Medicinal plants play a significant role in the development of potent therapeutic agents. Herbal medicines have been used to treat various human diseases. Moreover, the demand for herbal medicines is increasing day by day. *Punica granatum*, generally called as Pomegranate, is a deciduous tree belonging to the family Punicaceae. In Ayurveda, *P. granatum* is considered as "a pharmacy unto itself" and is used as an antiparasitic, antiviral, antifungal, antibacterial, hemostatic, anticarcinogenic agent and as a blood tonic. The most therapeutically beneficial pomegranate constituents are found to be ellagic acid, ellagitannins (punicalagins, punicalin, punicafolin), punicic acid, flavonoids, anthocyanidins, anthocyanins, flavonols, flavone glycosides and flavones.[9] In addition to this, *P. granatum* is one of the natural products having a potential hypoglycemic activity in which constituents like oleanolic, ursolic and gallic acids have been associated with antidiabetic effects.[10]

The leaves of *P. granatum* are known for their anti-inflammatory, anti-cholinesterase and cytotoxic activities.[11] Previous studies have reported the presence of high amounts of ellagic acid, an efficient free radical scavenger, in *P. granatum* leaf extract thus, demonstrating a potent antioxidant activity.[12] *P. granatum* leaves extract has been found to be highly effective in managing diabetic complications such as hyperlipidemia and thus, prevent the defects in lipid metabolism.[13] Further, the ethanolic extract of pomegranate leaves has shown a promising role as an anti-obesity agent in the high fat diet induced obesity model.[14] Taking all this into consideration, the objective of the present study was to assess the protective effects of methanolic extract of *P. granatum* leaves (MPGL) in streptozotocin (STZ)-induced diabetic nephropathy.

2. Material and methods

2.1. Procurement and authentication of plant

P. granatum leaves were collected from Nashik, Maharashtra; India in the month of January, 2014 and identified by Dr. Ganesh Iyer, Botany Department, Ruia College, Mumbai (Maharashtra). A voucher specimen (No. 2014/01) was deposited at the Department of Pharmacology, Institute of Chemical Technology, Mumbai (Maharashtra, India).

2.2. Preparation of plant extract

The leaves were washed with distilled water, shade dried and made into a coarse powder. The powder was initially defatted using petroleum ether followed by extraction with methanol using Soxhlet apparatus. The extract obtained was concentrated under reduced pressure using rotary evaporator (yield 50.707%) and stored in an airtight container for subsequent use.

2.3. Preliminary phytochemical screening

The phytochemical investigation of MPGL was carried out following the standard procedure of Wagner et al. (1996).[15] Plant extract was screened for the presence of steroids, triterpenoids, glycosides, flavonoids, saponins and tannins. The total phenolic content was quantified by calibration curve obtained by measuring the absorbance of the known concentrations of gallic acid standard solutions [10–100 μg/ml in methanol]. The results were calculated and expressed in mg of gallic acid equivalent (GAE) per gram of extract. The total flavonoid content was measured by the aluminium chloride colorimetric method and expressed as mg of rutin equivalents per gram of extract.[16]

2.4. Experimental

2.4.1. Animals

Healthy male Sprague Dawley rats weighing between 250 and300 g were procured from National Institute of Biosciences, Pune (Maharashtra) and maintained in polypropylene cages at ambient temperature of 22 ± 1 °C and relative humidity of 50–60% with a 12 h light/dark cycle in registered animal house (87/1999/CPCSEA) at Institute of Chemical Technology, Matunga, Mumbai. The animal experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the Institutional Animal Ethics Committee (IAEC), Institute of Chemical Technology, Mumbai, (Approval No. ICT/IAEC/2014/P38). Throughout the experimental period, the animals were fed with standard pellet diet (Amrut Brand, Sangali, India) and water *ad libitum*.

2.4.2. Experimental design

Diabetes was induced by a single injection of streptozotocin (STZ, 45 mg/kg, i.p. freshly prepared in 0.1 M citrate buffer pH 4.5) in rats.[17] The control group received equal volume of vehicle (0.1 M citrate buffer, pH 4.5). Blood glucose level was measured from tail vein using glucose meter (ACCU-CHEK advantage), seven days after induction. Rats with blood glucose level above 14 mmol/L were considered as diabetic and were used for further study by initiating the treatment. MPGL was administered daily to rats *per oral* for 8 weeks. The animals were divided into 5 groups: Normal control group (n = 6), STZ-induced diabetic group (n = 6), STZ-induced diabetic group treated with MPGL 100 mg/kg (n = 6), STZ-induced diabetic group treated with MPGL 200 mg/kg (n = 6) and STZ-induced diabetic group treated with MPGL 400 mg/kg (n = 6). The three doses of extract were selected on the basis of the acute oral toxicity study reported on the plant in addition to the previous studies carried out on the plant.[13,18] Blood glucose levels were measured before treatment and on 1st, 2nd, 4th, 6th & 8th weeks respectively. Body weight of each animal was determined at the initiation and end of the study. On the completion of 8 weeks, blood was withdrawn via retro orbital plexus. Blood samples were centrifuged at 1300*g* for separation of serum and stored at −20 °C until assay. At the end of the experimental period, animals were perfused with saline to remove the traces of blood cells from the organs.

2.5. Preparation of kidney homogenate

Immediately after sacrifice, both the kidneys were dissected; rinsed with isotonic saline and weighed. After weighing, each kidney was cut into two halves. One half was used for histopathological evaluation. Other half was minced and a homogenate was prepared with 10% (w/v) phosphate-buffered (0.1 M, pH 7.4) using a homogenizer. The kidney homogenate was centrifuged and the supernatant was estimated for kidney antioxidant parameters.

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2.6. Serum and urine parameters

Serum was used for the estimation of albumin, creatinine, blood urea nitrogen (BUN), cholesterol and triglyceride levels. Pooled 24 h urine was evaluated for creatinine and albumin value. The estimation of the above mentioned parameters was carried out using biochemical kits (ACCUREX, Biomedical Pvt. Ltd). For the determination of fluorescent AGE, a 96-plate spectrophotofluorimeter was employed. 100 μl of serum sample was placed in each well. Fluorescence intensity was read at 440 nm after excitation at 370 nm. Results were expressed as relative fluorescence intensity.

2.7. Kidney antioxidant parameters

5-5-dithiobis (2-nitrobenzoic acid) (DTNB) reagent was used to estimate reduced glutathione (GSH) level in tissue homogenates and the absorbance was read at 412 nm. The amount of GSH in the sample was calculated in microgram per ml from a standard curve obtained and represented in GSH per total tissue protein. Evaluation of kidney homogenate for lipid peroxidation levels, superoxide dismutase (SOD) and catalase (CAT) activities was carried out following the method published by Nishi et al., and Halliwell and Chirico.[19,20]

2.8. Kidney histopathological examination

Kidney tissues were immediately preserved in 10% neutral buffered formalin, dehydrated through graded alcohol series, embedded in paraffin, cut into 5 μm sections and stained with hematoxylin and eosin (H&E) and periodic acid Schiff base (PAS) method. The slides were examined by light microscopy under 400× magnification for microscopic alterations of pathological significance.

3. Results

3.1. Preliminary phytochemical screening

The preliminary phytochemical screening carried out on MPGL revealed the presence of phytoconstituents such as steroids, glycosides, saponins, flavonoids, tannins and carbohydrates (Fig. 1). The total phenolic content was found to be 328.75 ± 0.625 mg of gallic acid/g of extract whereas the total flavonoid content was found to be 126.667 ± 6.5 mg of rutin/g of extract respectively.

3.2. Body weight

During 8-week experiment, STZ-diabetic rats exhibited significant weight loss when compared with normal rats. At the end of 8 weeks treatment, the body weight of rats treated with MPGL at the dose of 200 mg/kg and 400 mg/kg was significantly increased compared with the diabetic control group (Table 1).

3.3. Kidney weight and kidney hypertrophy

The kidney weight and kidney hypertrophy index in STZ-diabetic rats were significantly increased as compared to those in the normal control group. Treatment of STZ-diabetic rats with MPGL at the dose of 200 mg/kg and 400 mg/kg ameliorated kidney hypertrophy index (Table 1).

3.4. Blood glucose level

Administration of STZ led to significant increase in fasting blood glucose levels in the diabetic group as compared to the normal control group throughout 8 weeks after induction. Furthermore, STZ-diabetic rats treated with 100 mg/kg MPGL demonstrated decrease in the fasting blood glucose levels at the end of 8 weeks whereas STZ-diabetic rats treated with 200 mg/kg and 400 mg/kg MPGL showed a significant decrease in the fasting blood glucose levels at the end of 6 weeks (Table 2).

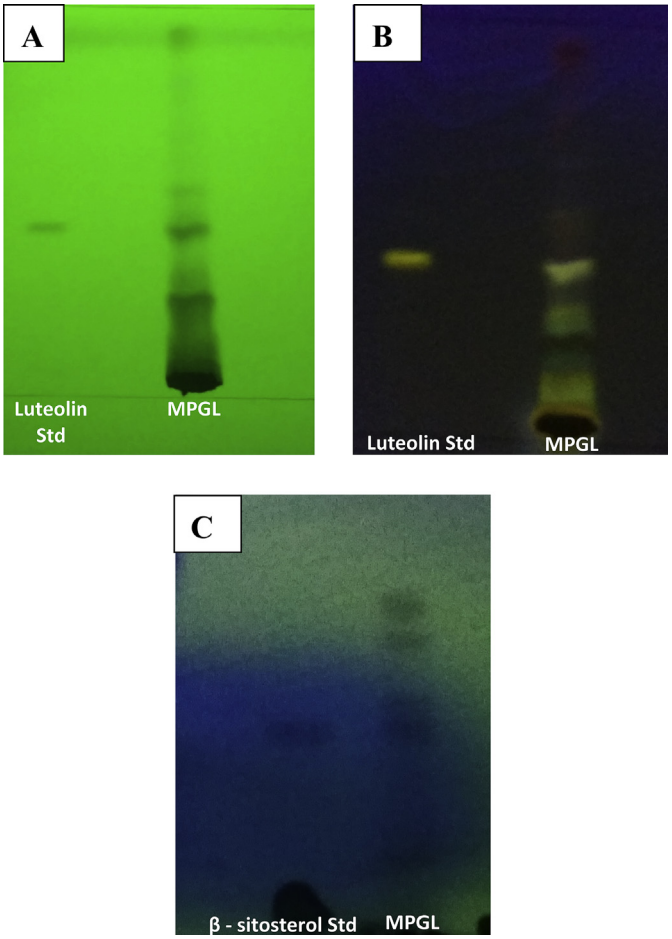

Fig. 1. TLC analysis of methanolic extract of *Punica granatum* leaves (MPGL). (A) Visualization of MPGL and Luteolin standard at 254 nm (Rf value: 0.37). (B) Visualization of MPGL and Luteolin standard at 366 nm (Rf value: 0.37). (C) Visualization of MPGL and β-sitosterol standard at 366 nm (Rf value: 0.46).

3.5. Serum and urine parameters

The STZ-diabetic rats showed a significant decrease in the level of creatinine in urine with a significant increase in serum as compared to the normal control group. After treatment with MPGL, the creatinine content increased significantly in urine with a significant decrease in serum. The STZ-diabetic rats showed a significant increase in the albumin content in urine, with a significant decrease in the level of albumin in serum as compared to the normal group. The albumin level of the STZ-diabetic rats treated with MPGL was brought back to normal. The STZ-diabetic rats exhibited significant increase in the blood urea nitrogen content whereas treatment with MPGL significantly decreased this level. In STZ-diabetic rats a significant increase in the level of serum triglycerides and cholesterol was observed as compared to the normal control group. However, STZ-diabetic rats treated with MPGL demonstrated a significant decrease in the level of triglycerides and cholesterol. Advanced glycation end products were significantly

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Table 1
Effect of *Punica granatum* leaves extract on body weight and kidney weight of STZ-diabetic rats.

Groups	Body weight (gm) 8th week Mean ± S.E.M.	Kidney weight (gm) Mean ± S.E.M.	Kidney hypertrophy (kidney weight/Body weight) %
Control	400.17 ± 9.82	1.2 ± 0.05	0.30 ± 0.01
Diabetic	217.83 ± 15.63###	1.43 ± 0.10#	0.60 ± 0.03###
100 mg/kg MPGL	231.33 ± 11.66	1.24 ± 0.05	0.54 ± 0.03
200 mg/kg MPGL	263.17 ± 9.02*	1.18 ± 0.05*	0.44 ± 0.02***
400 mg/kg MPGL	311.67 ± 10.72***	1.12 ± 0.06**	0.36 ± 0.02***

* Treatment group was compared with diabetic control, $p < 0.05$; ** Treatment group was compared with diabetic control, $p < 0.01$; *** Treatment group was compared with diabetic control, $p < 0.001$; ### Diabetic group was compared with normal control, $p < 0.001$, using one-way ANOVA with Dunnett's test.

Table 2
Effect of *Punica granatum* leaves extract on blood glucose level of STZ-diabetic rats.

	Blood glucose level (mmol/L) (Mean ± S.E.M.)					
Groups	After Induction	1st week	2nd week	4th week	6th week	8th week
Control	4.25 ± 0.33	4.26 ± 0.26	4.11 ± 0.33	4.34 ± 0.33	4.18 ± 0.33	4.29 ± 0.33
Diabetic	24.83 ± 3.04###	25.42 ± 3.22###	26.48 ± 1.47###	23.96 ± 1.93###	27.03 ± 2.61###	26.78 ± 1.55###
100 mg/kg MPGL	24.85 ± 4.00	24.41 ± 1.39	25.45 ± 1.66	23.33 ± 2.49	24.13 ± 1.88	20.14 ± 0.97*
200 mg/kg MPGL	25.86 ± 2.64	23.49 ± 3.79	17.89 ± 3.49*	23.43 ± 3.46	18.5 ± 2.11*	18.57 ± 2.11*
400 mg/kg MPGL	24.5 ± 2.51	20.85 ± 4.13	15.93 ± 1.11**	18.03 ± 1.76	17.81 ± 2.70*	16.86 ± 2.81**

* Treatment group was compared with diabetic control, $p < 0.05$; ** Treatment group was compared with diabetic control, $p < 0.01$; *** Treatment group was compared with diabetic control, $p < 0.001$; ### Diabetic group was compared with normal control, $p < 0.001$, using one-way ANOVA with Dunnett's test.

higher in the STZ-diabetic rats when compared with normal control rats. Administration of MPGL to STZ-diabetic rats reduced the advanced glycation end products significantly when compared to the negative control group (Tables 3 and 4).

3.6. Kidney antioxidant parameters

Significantly higher levels of Malondialdehyde (MDA) (a marker of lipid peroxidation) were detected in the kidney of STZ-diabetic rats. SOD, CAT and GSH activities were significantly decreased in the kidney of STZ-diabetic rats as compared to the normal control rats. Treatment with MPGL significantly decreased the increased level of MDA at the dose of 200 and 400 mg/kg and significantly increased the decreased activities of SOD and GSH at all dose levels. Significant elevation of catalase activity was observed at the dose of 400 mg/kg of MPGL (Fig. 2).

3.7. Histopathological evaluation

Control rats did not show any abnormal morphological changes in H&E and PAS stained kidney specimens. Normal architecture and glomerular size and basement membrane thickness were observed in case of control group. According to H&E stained kidney specimens of STZ-diabetic rats, moderate to severe vacuolar degeneration of tubules and increased glomerular space was observed. MPGL significantly reduced vacuolar degeneration of tubules at 200 mg/kg and 400 mg/kg dose levels but failed to demonstrate the same at 100 mg/kg (Fig. 3). Kidneys stained with PAS exhibited moderate intensity PAS positivity in glomeruli and mild degree basement membrane thickening in the negative control group. Treatment with MPGL reduced the PAS positivity staining intensity in glomeruli at all dose levels and basement membrane thickening in STZ-diabetic rats at 400 mg/kg dose level respectively (Fig. 4).

Table 4
Effect of *Punica granatum* leaves extract on urine parameters of STZ-diabetic rats.

Groups	Urine albumin gm/24 h	Urine creatinine mg/24 h
	(Mean ± S.E.M.)	
Control	0.09 ± 0.01	22.90 ± 0.41
Diabetic	0.40 ± 0.03###	12.53 ± 0.23###
100 mg/kg MPGL	0.22 ± 0.06**	14.31 ± 0.86
200 mg/kg MPGL	0.22 ± 0.03**	16.59 ± 0.05***
400 mg/kg MPGL	0.16 ± 0.02***	21.73 ± 0.55***

* Treatment group was compared with diabetic control, $p < 0.05$; ** Treatment group was compared with diabetic control, $p < 0.01$; *** Treatment group was compared with diabetic control, $p < 0.001$; ### Diabetic group was compared with normal control, $p < 0.001$, using one-way ANOVA with Dunnett's test.

Table 3
Effect of *Punica granatum* leaves extract on serum parameters of STZ-diabetic rats.

Groups	Serum albumin gm %	Serum creatinine mg/dl	BUN mg/dl	Total Cholesterol mg %	Triglycerides mg %	Serum AGE RFU
			(Mean ± S.E.M.)			
Control	6.37 ± 0.02	1 ± 0.12	15.89 ± 2.84	56.31 ± 3.15	77.87 ± 11.42	14726.88 ± 646.37
Diabetic	2.46 ± 0.15###	1.47 ± 0.03##	32.62 ± 2.12###	77.31 ± 8.12#	151.38 ± 3.89###	25619.980 ± 622.98###
100 mg/kg MPGL	2.20 ± 0.42	1.15 ± 0.17	23.00 ± 0.27**	63.85 ± 3.84	129.45 ± 8.65	25092.75 ± 297.75
200 mg/kg MPGL	3.66 ± 0.60	0.95 ± 0.05**	21.62 ± 0.99***	53.63 ± 3.55**	81.83 ± 9.24***	21275.3 ± 356.7***
400 mg/kg MPGL	4.55 ± 0.36**	0.80 ± 0.00***	17.73 ± 1.21***	51.16 ± 3.18**	78.94 ± 2.92***	20028.33 ± 419.83***

* Treatment group was compared with diabetic control, $p < 0.05$; ** Treatment group was compared with diabetic control, $p < 0.01$; *** Treatment group was compared with diabetic control, $p < 0.001$; ### Diabetic group was compared with normal control, $p < 0.001$, using one-way ANOVA with Dunnett's test.

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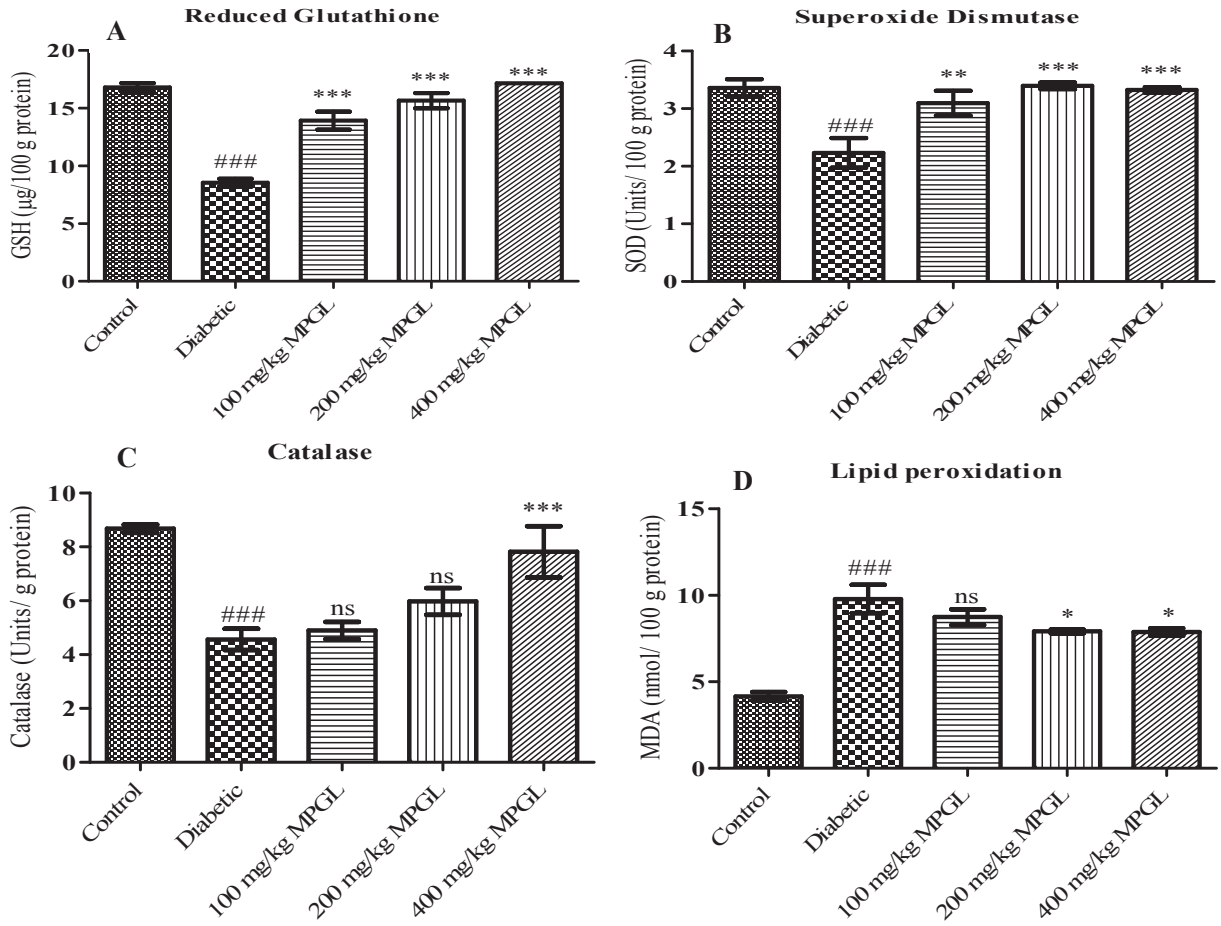

Fig. 2. Effect of *Punica granatum* leaves extract on antioxidant parameters in STZ-diabetic rats. (A) GSH in kidney homogenate of control, STZ-diabetic rats and MPGL treated rats. (B) SOD in kidney homogenate of control, STZ-diabetic rats and MPGL treated rats. (C) Catalase in kidney homogenate of control, STZ-diabetic rats and MPGL treated rats. (D) Lipid peroxidation in kidney homogenate of control, STZ-diabetic rats and MPGL treated rats. * Treatment group was compared with diabetic control, $p < 0.05$; ** Treatment group was compared with diabetic control, $p < 0.01$; *** Treatment group was compared with diabetic control, $p < 0.001$; ### Diabetic group was compared with normal control, $p < 0.001$, using one-way ANOVA with Dunnett's test.

4. Discussion

Diabetes is a complex endocrine disorder involving insulin deficiency which leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism. It is estimated that more than 300 million people worldwide will have diabetes mellitus by 2025.[21] Diabetic nephropathy, a serious complication of diabetes mellitus, is the most common cause of end stage renal failure. About 15–25% of type 1 diabetes patients and 30–40% of patients with type 2 diabetes suffer from diabetic nephropathy.[22] In spite of the availability of therapeutic agents which retard the progression of diabetic nephropathy, there has been renewed interest in the use of herbal medicines in order to prevent the genesis of this complication.

P. granatum L., a deciduous shrub, the leaves of which are rich in polyphenolic compounds including tannins and flavonoids[23] has aroused great interest due to its antidiabetic potential which could be considered as a lead to further study the effect of this part of the plant on diabetic complications such as nephropathy.[24] Taking this into consideration, in our present investigation, we have evaluated the protective effects of MPGL on STZ-induced diabetic nephropathy in rats.

Streptozotocin has been an agent of choice to induce experimental diabetes mellitus due to its ability to induce specific necrosis of the pancreatic beta cells resulting in degranulation and loss of capacity to secrete insulin.[25,26] Thus, in the present study, STZ was used for induction of diabetes in rats. Administration of STZ led to significant increase in blood glucose level which was lowered on treatment with MPGL thus confirming the antihyperglycemic activity of the extract as reported by earlier established studies.[13,24] However, up to 4 weeks no significant difference was observed in the glucose level of MPGL treated groups as compared to diabetic group. One of the possible reasons for this may be that the extract does not possess a strong anti-diabetic activity. The concentration of active principles at the given doses might not be capable in controlling hyperglycemia in the initial weeks. The active principles in the extract might take time to reach sufficient concentration at the target site. It could also be due to principles such as reducing sugars or other carbohydrates which might interfere with the onset of hypoglycemic effect of MPGL. Several reports have stated the diminished hypoglycemic responses of extract due to the presence of reducing sugars which could give rise to free glucose after digestion that may tend to rise blood glucose levels in the face of the hypoglycemic actions by the active hypoglycemic agents.[27]

STZ-induced diabetes is associated with significant reduction in the body weight due to hyperglycemia, hypoinsulinemia,[25] increased muscle wasting and loss of tissue proteins.[28] The body weight of STZ-diabetic rats was progressively reduced and treatment with MPGL improved the body weight significantly, thus indicating prevention of muscle tissue damage caused due to hyperglycemia.

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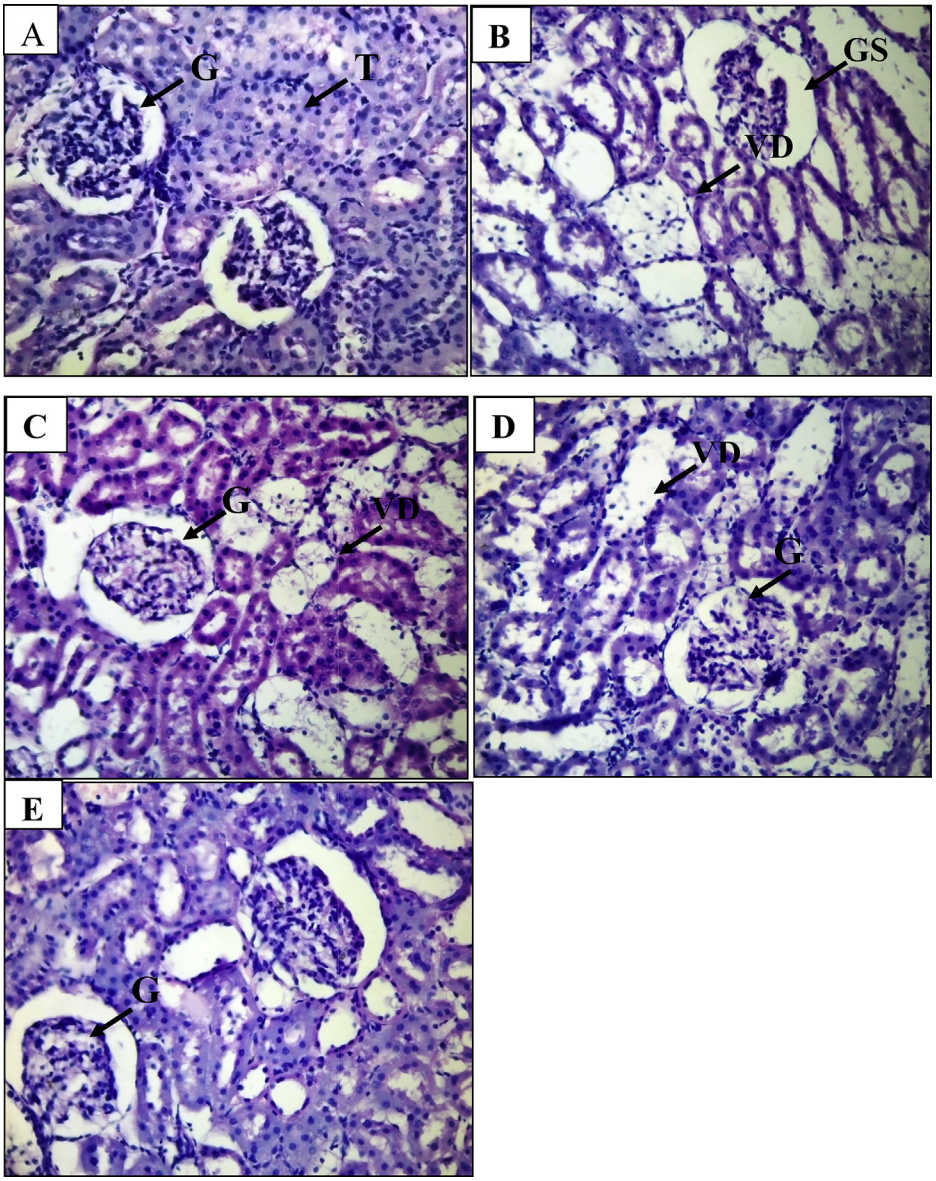

Fig. 3. Photomicrographs of kidney (Hematoxylin and Eosin staining under a light microscope at 400× magnification). A: Control rats; B: STZ-diabetic rats; C: 100 mg/kg MPGL treated group; D: 200 mg/kg MPGL treated group; E: 400 mg/kg MPGL treated group. G: glomeruli, T: tubules, GS: glomerular space, VD: vacuolar degeneration.

Increase in the weight of kidney (hypertrophy) in proportion to the body weight is observed in STZ-induced rats. Local alterations in the production of one or more growth factors such as over expression of transforming growth factor – beta 1 in the kidney especially in proximal convoluted tubules cells and glomerular mesangial cells are proposed in the development of renal hypertrophy. An increase in the rate of protein synthesis and/or decrease in the degradation of renal extracellular components might also lead to renal hypertrophy.[25] MPGL treatment reduced kidney/body weight ratio, thus demonstrating reversal of kidney hypertrophy in STZ-diabetic rats.

Diabetes mellitus leads to fatty liver, hypercholesterolemia and hypertriglyceridemia.[24] Moreover, elevated cholesterol levels are associated with diabetic nephropathy.[29] These increased levels were reversed towards normal after treatment with MPGL, thus demonstrating its potential to improve lipid metabolism. Hypoalbuminaemia is considered as a strongest predictor of death in patients with renal failure. Albumin is by far the most abundant protein in nephrotic urine.[30] In STZ-diabetic rats, serum albumin concentration was decreased significantly with an increase in the albumin levels in urine, thus demonstrating that albuminuria was related to deteriorating kidney function. Treatment with MPGL normalized these levels thus, exhibiting its beneficial role against microalbuminuria. Increased serum creatinine level and BUN along with decreased excretion of creatinine in the urine are indicators of the development of diabetic nephropathy.[22] Reversal of these effects was observed in STZ-diabetic rats treated with MPGL. The formation of AGEs in renal tissue plays a crucial role in the development of diabetic nephropathy. The irreversible formation of AGEs affects proteins and lipids thus causing damage to the blood vessels and kidneys.[31] AGEs are found in almost all tissues examined from STZ-induced diabetic rats. Moreover, kidneys are more susceptible to AGE formation than other tissues.[32] Increased level of AGE was found in the serum of STZ-diabetic rats whereas treatment with MPGL lowered the elevated AGE levels. Thus MPGL exhibited the potential to protect the kidney by decreasing the formation of AGE in the circulation of the STZ-diabetic rats.

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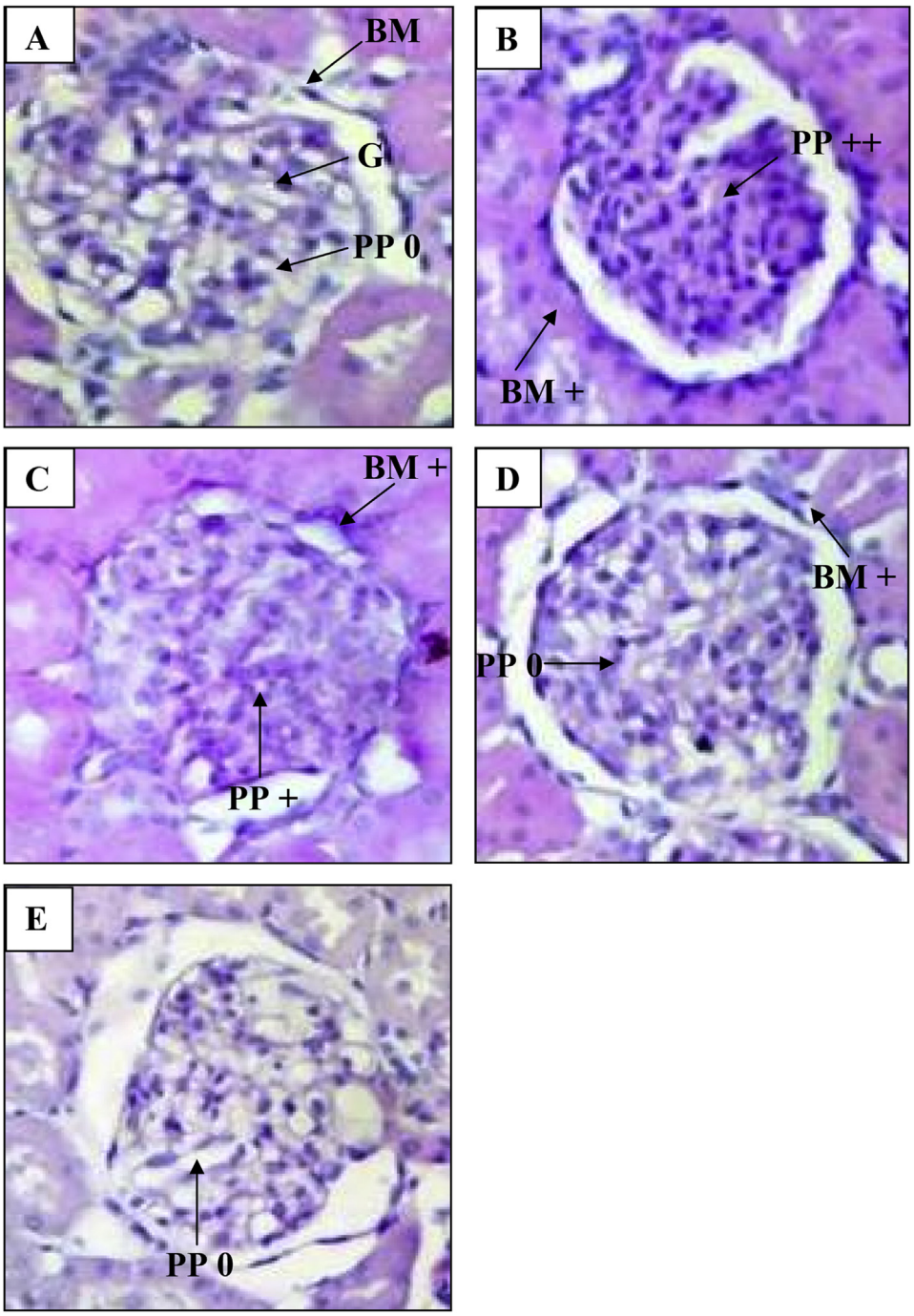

Fig. 4. Photomicrographs of kidney (Periodic Acid Schiff staining under light microscope at 400× magnification). A: Control rats; B: STZ-diabetic rats; C: 100 mg/kg MPGL treated group; D: 200 mg/kg MPGL treated group; E: 400 mg/kg MPGL treated group. PP: PAS positivity, BM: Basement membrane thickening, G: glomeruli, +: mild change, ++: moderate change.

Hyperglycemia leads to increased production of reactive oxygen species (ROS) which are involved in the etiology of several diabetic complications including diabetic nephropathy.[28,33] The reactive oxygen species deplete the antioxidant defenses of the cell thus making it more susceptible to oxidative damage. It further targets lipid, DNA and protein leading to their oxidation which further leads to changes in cellular structure and function. GSH plays a crucial role as a free radical scavenger in addition to the maintainance of plasma antioxidant status. Superoxide dismutase converts superoxide to a less reactive ROS, hydrogen peroxide which is further reduced to water by catalase. Thus, catalase assists SOD in the complete neutralization of ROS.[32] MDA, a late-stage lipid oxidation byproduct, is an important indicator of free radical-induced lipid peroxidation.[28] Increased MDA levels are found in mesangial cells, proximal tubule cells, plasma and renal cortex.[32] Increased MDA levels were suppressed in STZ-diabetic rats treated with MPGL. In addition to this, STZ-diabetic rats treated with MPGL showed increased levels of GSH, SOD and catalase, thus suggesting the antioxidant capacity of MPGL.

Histopathological examination of kidney sections of STZ-diabetic rats showed severe vacuolar degeneration of tubules, increased glomerular space, moderate intensity PAS positivity in

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glomeruli and basement membrane thickening. Treatment with MPGL significantly reduced the aforementioned alterations, thus demonstrating protective role in renal damage.

5. Conclusions

In the present investigation, administration of MPGL to STZ-diabetic rats decreased blood glucose level, normalized the cholesterol and triglycerides level, ameliorated the serum and urine parameters along with normalization of kidney antioxidant status.

In summary, MPGL possesses antioxidant, antihyperglycemic, antihyperlipidemic and anti-glycation activity and thus, exhibits a protective action in STZ-induced diabetic nephropathy. Moreover, further work is necessary to elucidate in detail the mechanism of action of MPGL at the cellular and molecular levels.

Conflicts of interest statement

The authors declare no conflict of interest.

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